Effects of two novel tachykinin antagonists, FK224 and FK888, on neurogenic airway plasma exudation, bronchoconstriction and systemic hypotension in guinea-pigs *in vivo*

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1 We compared the effects of two novel tachykinin receptor antagonists, FK888 (selective at the tachykinin NK₁ receptor) and FK224 (dual antagonist at NK₁ and NK₂ tachykinin receptors) on stimulus-evoked airway plasma exudation, bronchoconstriction and systemic hypotension in guinea-pigs *in vivo*. Plasma exudation was induced by substance P (SP), synthetic tachykinin receptor agonists, platelet activating factor (PAF), electrical stimulation of the cervical vagus nerves or by inhalation of cigarette smoke. Changes in airway tone and in carotid artery blood pressure (BP) were induced by synthetic tachykinin agonists, PAF and vagal stimulation.

2 Both FK224 and FK888 dose-dependently inhibited SP-induced plasma exudation in the lower trachea and main bronchi (ID₅₀ values respectively of 1.1 and 0.1 $\mu\text{mol kg}^{-1}$ in lower trachea, and of 0.5 and 0.1 $\mu\text{mol kg}^{-1}$ in main bronchi) with complete inhibition at both airway levels at 10 $\mu\text{mol kg}^{-1}$ for FK224 and at 2 $\mu\text{mol kg}^{-1}$ for FK888.

3 The NK₁-selective tachykinin receptor agonist, [Sar⁹,Met(O₂)¹¹]substance P ([Sar]SP), induced plasma exudation, a response which was blocked by both FK888 and FK224. The NK₂-selective agonist, [β -Ala⁶]neurokinin A-(4-10) ([β -Ala]NKA), did not induce plasma exudation: neither FK888 nor FK224 affected this lack of response to [β -Ala]NKA.

4 [β -Ala]NKA induced bronchoconstriction, a response which was blocked by FK224 but which was completely unaffected by FK888. [Sar]SP induced a small but significant bronchoconstriction which was completely inhibited by both tachykinin antagonists.

5 In animals pretreated with capsaicin to deplete sensory neuropeptides, PAF induced both plasma exudation and bronchoconstriction. Neither response to PAF was inhibited by either FK888 or FK224.

6 Both FK888 and FK224 inhibited plasma exudation induced by vagus nerve stimulation or by cigarette smoke, with FK888 more potent than FK224.

7 FK224 inhibited non-cholinergic bronchoconstriction induced by vagal stimulation, whereas FK888, at doses inhibiting vagally-induced plasma exudation, did not.

8 Decreases in BP induced by SP or [Sar]SP were blocked by both FK888 and FK224. In contrast, neither antagonist had any significant inhibitory effect on the decrease in BP induced by vagal stimulation (in the presence of atropine) or PAF. [β -Ala]NKA did not decrease BP and neither tachykinin antagonist had any significant effect on this lack of response.

9 We conclude that in guinea-pig airways, plasma leakage induced by endogenous tachykinins is mediated predominantly via NK₁-receptors, whereas bronchoconstriction is mediated predominantly via NK₂-receptors. In addition, SP-evoked decreases in BP are also mediated via NK₁ receptors, whereas the contribution of endogenous tachykinins to vagally-induced decreases in BP appears to be minimal. Development of selective tachykinin receptor antagonists will be important in understanding the involvement of tachykinins in airway physiology and pathophysiology, whereas potent dual tachykinin receptor antagonists such as FK224 may have greater therapeutic potential in certain airway diseases in which tachykinins have been implicated in pathogenesis, including asthma and chronic bronchitis associated with cigarette smoking.

Keywords: FK224; FK888; tachykinin antagonist; NK₁-receptor; NK₂-receptor; plasma exudation; bronchoconstriction; vagus nerve; cigarette smoke; asthma

Introduction

Three types of tachykinin receptor (i.e. NK₁, NK₂, NK₃) are currently recognized, at which substance P (SP), neurokinin A (NKA) or NKB respectively are thought to be the most relevant natural agonists (Guard & Watson, 1991). Among these, SP and NKA may be more important in certain lung diseases, including asthma, in terms of their possible role in neurogenic inflammation and smooth muscle contraction (Barnes *et al.*, 1991).

Electrical stimulation of the cervical vagus nerves, in the presence of atropine, in experimental animals leads to airway plasma exudation and non-cholinergic bronchoconstriction, responses which are mediated via neuropeptides released from capsaicin-sensitive sensory nerves (Lundberg *et al.*, 1983b). Cigarette smoke also induces plasma exudation, again induced by endogenously released neuropeptides from sensory nerves (Lundberg *et al.*, 1983a). In guinea-pig airways *in vivo* the order of potency of SP > NKA > NKB in causing plasma leakage (Rogers *et al.*, 1988) indicates that NK₁-receptors mediate the response, a suggestion consistent with inhibition of neurogenic plasma exudation by a selective

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non-peptide NK₁-antagonist, CP-96,345 (Delay-Goyet & Lundberg, 1991; Eglezos *et al.*, 1991; Lei *et al.*, 1992). In contrast, in guinea-pig airways *in vitro* agonist potencies (Advenier *et al.*, 1987) and the activity of synthetic antagonists in inhibiting non-cholinergic neurogenic airway contraction (Maggi *et al.*, 1991b) suggest a predominant role for NK₂ receptors mediating smooth muscle constriction with a smaller contribution from NK₁ receptors. In human bronchus *in vitro*, agonist potencies indicate that only NK₂ receptors mediate bronchoconstriction (Advenier *et al.*, 1987).

In the present study, we compared the effects of two new tachykinin receptor antagonists, FK224 and FK888 (Figure 1), on plasma exudation induced by exogenous SP, the synthetic tachykinin agonists [Sar⁹,Met (O₂)¹¹]substance P ([Sar]SP) and [β-Ala⁸]neurokinin A-(4-10) ([β-Ala]NKA) which are selective for the NK₁ and NK₂ receptor respectively (Maggi *et al.*, 1990a,b), platelet activating factor (PAF), electrical stimulation of the vagus nerves or by inhalation of cigarette smoke. FK224 is a dual antagonist at both NK₁ and NK₂ receptors (Morimoto *et al.*, 1992; Murai *et al.*, 1992), whereas FK888 is a highly selective antagonist at NK₁ receptors (Fujii *et al.*, 1992). We also compared the effects of both compounds on bronchoconstriction and hypotension induced by [β-Ala]NKA, [Sar]SP, PAF and vagal stimulation. The airway effects of PAF may be mediated via sensory nerve activation and release of sensory neuropeptides including SP and NKA (Martins *et al.*, 1991). Consequently, we used animals pretreated with capsaicin (Holzer, 1991) to eliminate sensory neuropeptide involvement in the studies with PAF.

Methods

Animal preparation

Male Dunkin-Hartley outbred guinea-pigs (Charles River U.K. Ltd., Kent) weighing 260–440 g were anaesthetized

with urethane (2 g kg⁻¹, i.p.). A tracheal cannula was inserted via a tracheostomy into the lumen of the trachea and connected to a constant-volume mechanical ventilator (Harvard Apparatus Limited, Edenbridge, Kent). A tidal volume of 10 ml kg⁻¹ and a frequency of 60 breaths min⁻¹ were used to ventilate the animals. Pulmonary insufflation pressure (PIP) was measured by means of a pressure transducer (Sensortech, Rugby, Warwks.) connected to a side arm in the expiratory limb of the ventilation tubing. Blood pressure was monitored throughout the experiments via a polyethylene catheter filled with heparin-saline (10 u ml⁻¹) inserted into the left carotid artery and linked to a pressure transducer (Bell and Howell, Basingstoke) and two channel recorder (Ormed Ltd., Welwyn Garden City). All drugs were administered via the jugular veins.

Measurement of plasma exudation

To assess plasma exudation, Evans blue dye (25 mg kg⁻¹) was used as a plasma marker (Rogers *et al.*, 1989b) and was injected 1 min before each experimental procedure. Five min after vagus nerve stimulation or 15 min after SP or cigarette smoke, the systemic circulation of the animals was perfused with saline via the aorta to expel intravascular dye. The lower half of the trachea and the main bronchi were removed and the Evans blue dye in the tissue was extracted in formamide (37°C for 16 h) and quantified spectrophotometrically by measuring dye absorbance at 620 nm wavelength. Tissue dye content was expressed as ng dye mg⁻¹ wet weight tissue.

Protocol

In the experiments involving vagus nerve stimulation, animals were first given atropine (1 mg kg⁻¹). After 8 min, both cervical vagus nerves were cut at the level of the fifth tracheal cartilage ring. Two min later, the caudal ends of the nerves were stimulated with bipolar platinum electrodes at 5 V, 5 ms, 10 Hz for 3 min (Belvisi *et al.*, 1990). Control animals were prepared similarly but without electrical stimulation (sham stimulation).

The cigarettes used were commercially available unfiltered U.K. government category 'Middle Tar' (nicotine content 1.2 mg per cigarette, carbon monoxide content 11 mg per cigarette) and were lit in a fume cupboard where a laminar flow prevented smoke accumulation. Smoke was collected into a 50 ml-syringe and blown into the trachea via 3-way tap immediately rostral to the endotracheal tube. One tidal volume of cigarette smoke was given every five to six ventilated breaths of room air. Control animals inhaled air drawn through an unlit cigarette.

In the experiments involving PAF, one group of animals were pretreated with capsaicin (or its vehicle) to deplete sensory neuropeptides. To reduce capsaicin-induced bronchoconstriction, animals were given aminophylline (25 mg kg⁻¹, i.p.) and terbutaline (0.1 mg kg⁻¹, s.c.). These administrations were repeated 20 min later. Five min after the second administration of bronchodilators, the animals were anaesthetized with xylazine (0.1 mg kg⁻¹, i.m.) and ketamine hydrochloride (50 mg kg⁻¹, i.m.) and injected with capsaicin (50 mg kg⁻¹, s.c.) or equivalent volume of vehicle (10% ethanol and 10% Tween 80, v v⁻¹ in saline).

SP (1 nmol kg⁻¹), [Sar]SP (1 nmol kg⁻¹) or [β-Ala]NKA (10 nmol kg⁻¹) (Maggi *et al.*, 1991a) or their vehicle (saline), or C₁₆-PAF (5 µg kg⁻¹) or its vehicle (0.5% ethanol in saline) were injected intravenously 1 min after Evans blue dye. Tachykinin antagonists or their vehicle (dimethylsulphoxide, DMSO; see Drugs below) were injected 5 min before SP, [Sar]SP, [β-Ala]NKA, PAF, vagus nerve stimulation or administration of cigarette smoke.

Figure 1 The structures of FK224 and FK888.

Drugs

The following drugs were used: SP, DMSO, formamide, C₁₆-PAF, capsaicin and urethane (Sigma Chemical Co. Ltd., Poole, Dorset), [Sar]P (Peninsula Labs., Merseyside), atropine sulphate (Phoenix Pharmaceuticals Ltd., Gloucester), Evans blue dye (Aldrich Chemical Co. Ltd., Gillingham, Kent), saline (0.9% sodium chloride BP for intravenous infusion, FL Manufacturing Ltd., Basingstoke). [β -Ala]NKA was a gift from A. Menarini Pharmaceuticals, Florence, Italy. Stock solutions of 0.1 $\mu\text{mol ml}^{-1}$ SP, of 3 $\mu\text{mol ml}^{-1}$ [Sar]SP and [β -Ala]NKA in saline, and of 1 mg ml^{-1} PAF in ethanol were each stored at -20°C and diluted in saline on each day of experimentation.

FK224 and FK888 were provided by the Fujisawa Pharmaceutical Co. Ltd. (Osaka, Japan). FK224 is a modified cyclic peptide with the structure N-(N²-(N-(N-(2,3-didehydro-N-methyl-N-(N-(3-(2-pentylphenyl)-propionyl)-L-threonyl)tyrosyl-L-leucynyl)-D-phenylalanyl)-L-allo-threonyl)-L-asparaginy-L-serine-D-lactone. FK888 is a synthetic

dipeptide with the structure N²-[(4R)-4-hydroxy-1-(1-methyl-1H-indol-3-yl)carbonyl-L-prolyl]-N-methyl-N-phenylmethyl-3-(2-naphthyl)-L-alaninamide. Both antagonists were dissolved in DMSO (20 $\mu\text{mol ml}^{-1}$ for FK888, 100 $\mu\text{mol ml}^{-1}$ for FK224), aliquoted and stored at 4°C .

In a number of instances the effect of DMSO on plasma exudation, bronchoconstriction and hypotension was compared with no administration. DMSO significantly ($P < 0.05$) affected the magnitude of plasma exudation compared with no treatment in air controls and sham-stimulated controls: the percentage difference between the mean values for DMSO and no DMSO respectively for each group was 42% and 8% for the lower trachea and 19% and 30% for the main bronchi. DMSO affected the magnitude of cigarette smoke or vagally stimulated plasma exudation to a lesser degree (maximum percentage difference between the means of 12%). Bronchoconstriction was 4% higher in sham-stimulated animals and 11% higher in stimulated animals with DMSO compared with no treatment. Blood pressure was minimally affected by DMSO (maximum percentage difference between mean values for 7 pairs of experimental groups was 5%). Thus, to account for the effects of DMSO in each study in Results involving the antagonists, DMSO, at the appropriate dilution in saline for the highest dose of antagonist used, was administered at appropriate time-points to both stimulated and unstimulated animals.

Data analyses

Data in the Results section are mean and one s.e.mean. Increases in PIP induced by vagus nerve stimulation were expressed as percentage increases for the peak of the response above the pre-stimulation value. Mean blood pressure was calculated from the recorded traces as diastolic pressure + 0.33 (systolic pressure – diastolic pressure). Decreases in mean BP were expressed as a percentage of the mean BP just before nerve stimulation or drug administration. The dose of antagonist causing 50% inhibition (ID_{50}) was calculated by non-linear regression. The significance of differences between groups was assessed by the Mann-Whitney U-test. The null hypothesis was rejected at $P < 0.05$ (two-tail test). Inhibition was considered to be complete when the response to stimulation in the presence of antagonist was significantly different from the response to stimulation without the antagonist and not significantly different from unstimulated controls.

Results

Effects of FK224 and FK888 on plasma exudation

The inhibitory effects of FK224 and FK888 were initially determined on SP-induced plasma exudation in the lower trachea and main bronchi (Figure 2). Both compounds gave a dose-dependent inhibition and, at the highest doses used (10 $\mu\text{mol kg}^{-1}$ FK224 or 2 $\mu\text{mol kg}^{-1}$ FK888), totally abolished the response. FK888 was 5–10 fold more potent than FK224 in inhibiting the response; ID_{50} values for FK224 and FK888 were respectively 1.1 and 0.1 $\mu\text{mol kg}^{-1}$ (1.10 and 0.06 mg kg^{-1}) in lower trachea, and 0.5 and 0.1 $\mu\text{mol kg}^{-1}$ (0.51 and 0.06 mg kg^{-1}) in main bronchi.

[Sar]SP (1 nmol kg^{-1}) significantly increased plasma exudation by 185% above saline controls (Figure 3). Both FK888 (2 $\mu\text{mol kg}^{-1}$) and FK224 (10 $\mu\text{mol kg}^{-1}$) abolished [Sar]SP-induced plasma exudation (Figure 3). In contrast, [β -Ala]NKA did not induce significant plasma exudation (Figure 3): neither antagonist significantly affected this lack of response ($n = 5$ for each antagonist; data not shown).

In animals treated one week previously with the vehicle for capsaicin or with capsaicin itself, PAF increased plasma exudation in the main bronchi (Table 1). The magnitude of PAF-induced plasma exudation was 21% greater in vehicle-

Figure 2 Effects of FK224 (a) and FK888 (b) on substance P (SP 1 nmol kg^{-1})-induced plasma exudation *in vivo* in guinea-pig lower trachea (solid symbols) or main bronchi (open symbols): ($\square$, $\blacksquare$) DMSO (vehicle for FK compounds) + saline; (Δ , $\blacktriangle$) DMSO + SP (1 nmol kg^{-1}); ($\circ$, $\bullet$) FK224 or FK888 + SP. All drugs given intravenously. Data are expressed as mean tissue content of the plasma marker Evans blue dye for 5–10 animals; s.e.mean shown by vertical bars. * $P < 0.05$; ** $P < 0.01$ vs DMSO + SP.

Figure 3 Effects of FK888 ($2 \mu\text{mol kg}^{-1}$) and FK224 ($10 \mu\text{mol kg}^{-1}$) on plasma exudation induced *in vivo* in guinea-pigs by [Sar]SP (NK_1 agonist, 1 nmol kg^{-1}). * $P < 0.05$ compared with control. The NK_2 -selective agonist $[\beta\text{-Ala}] \text{NKA}$ (10 nmol kg^{-1}) did not induce significant plasma exudation. All drugs given intravenously. Data are expressed as mean tissue content of the plasma marker Evans Blue dye for 5 animals; s.e.mean shown by vertical bars.

pretreated compared with capsaicinized animals, although there was no significant difference between the two groups. Neither FK888 nor FK224 inhibited PAF-induced plasma exudation (Table 1).

Electrical stimulation of the vagus nerves or cigarette smoke inhalation induced significant increases in the tissue content of Evans blue dye in both the lower trachea (by 165–292% above controls) and main bronchi (the latter shown in Figure 4). Both FK224 and FK888 inhibited these responses. FK224 at $1 \mu\text{mol kg}^{-1}$ or FK888 at $0.2 \mu\text{mol kg}^{-1}$ completely abolished the increase in plasma exudation induced by vagus nerve stimulation in both the lower trachea and main bronchi (Figure 4). FK224 and FK888 partially but significantly ($P < 0.05$) inhibited the cigarette smoke-induced response in the lower trachea (by approximately 50% at the highest dose of each compound used) and induced complete inhibition in the main bronchi (Figure 4).

Effects of FK224 and FK888 on bronchoconstriction

$[\beta\text{-Ala}] \text{NKA}$ increased pulmonary insufflation pressure by 259% above baseline (Figure 5). FK888 did not significantly affect this response (in fact induced a potentiation of 39%), whereas FK224 inhibited $[\beta\text{-Ala}] \text{NKA}$ -induced bronchocon-

Figure 4 Effects of FK224 (a) and FK888 (b) on vagus nerve stimulation or cigarette smoke-induced plasma exudation *in vivo* in main bronchi of guinea-pigs. Open column, control; solid column, response to stimulation; hatched and cross-hatched columns, effects of FK224 or FK888. All drugs given intravenously. Data are expressed as mean tissue content of the plasma marker Evans blue dye for 5 or 6 animals; s.e.mean shown by vertical bars. * $P < 0.05$ vs stimulation.

striction by 87% (Figure 5). [Sar]SP induced a small (16% above baseline) but significant increase in PIP, a response that was inhibited by 95% by FK888 and by 100% by FK224 (Figure 5).

In capsaicin or vehicle pretreated animals, PAF-induced increases in PIP were greater (although not significantly so) by 24% in vehicle-pretreated compared with capsaicinized animals. PAF-induced bronchoconstriction was not significantly affected by either of the tachykinin receptor antagonists under study (Table 1).

Electrical stimulation of the vagus nerves caused non-cholinergic bronchoconstriction as well as plasma exudation (Figure 6). The increase in PIP reached a peak approximately 1 min after the start of stimulation. FK224 induced a significant inhibition of 89% at $1 \mu\text{mol kg}^{-1}$ (Figure 6)

Table 1 Effect of tachykinin receptor antagonists on plasma exudation and bronchoconstriction induced by platelet activating factor (PAF)

	[Evans blue dye] (ng mg ⁻¹ wet wt. tissue)	PIP (cmH ₂ O)
Vehicle for capsaicin + DMSO + PAF	212.0 ± 20.2	328.8 ± 32.4
Capsaicinization + DMSO + PAF	175.9 ± 30.2	264.7 ± 49.1
Capsaicinization + FK888 + PAF	164.0 ± 12.7	305.0 ± 39.4
Capsaicinization + FK224 + PAF	175.7 ± 34.0	299.2 ± 41.7

Guinea-pigs were pretreated with capsaicin, to deplete sensory neuropeptides, or its vehicle. One week later animals were anaesthetized and given PAF ($5 \mu\text{g kg}^{-1}$) to induce exudation of the plasma marker Evans blue dye into the main bronchi and to increase pulmonary insufflation pressure (PIP), a measure of airway patency, either with the tachykinin antagonists FK888 ($2 \mu\text{mol kg}^{-1}$) or FK224 ($10 \mu\text{mol kg}^{-1}$), or their vehicle (dimethylsulphoxide, DMSO). All drugs given intravenously. Data are mean $\pm$ s.e.mean for 5 animals per group. There were no significant differences between treatment groups for plasma exudation or PIP.

Figure 5 Effect of FK888 ($2 \mu\text{mol kg}^{-1}$) and FK224 ($10 \mu\text{mol kg}^{-1}$) on the increase in pulmonary insufflation pressure (PIP), a measure of airway patency, induced *in vivo* in guinea-pigs by the NK_2 agonist, $[\beta\text{-Ala}]\text{NKA}$ (10 nmol kg^{-1}) (a) or the NK_1 agonist, $[\text{Sar}]\text{SP}$ (1 nmol kg^{-1}) (b). All drugs given intravenously. Data are expressed as mean for 5 animals; s.e.mean shown by vertical bars. * $P < 0.05$ compared with baseline; † $P < 0.05$ compared with value for agonist (with antagonist vehicle, dimethylsulphoxide (DMSO)).

whereas FK888 showed no significant effect on the response even at $0.2 \mu\text{mol kg}^{-1}$, the dose giving complete inhibition of neurogenic plasma exudation induced by vagus nerve stimulation.

Cigarette smoke did not induce bronchoconstriction, and this lack of response was not affected by either FK888 or FK224 (data not shown).

Effects of FK224 and FK888 on decreases in blood pressure

Administration of 1 nmol kg^{-1} SP decreased BP in the carotid artery with a maximal fall approximately 1 min after

Figure 6 Effects of FK224 or FK888 on vagus nerve stimulation-induced increases in pulmonary insufflation pressure (PIP), a measure of airway patency, *in vivo* in guinea-pigs. Open columns, control; solid column, response to stimulation; hatched and cross-hatched columns, effects of FK224 or FK888. All drugs given intravenously. Data are expressed as mean for 5 animals; s.e.mean shown by vertical bars. * $P < 0.05$ vs stimulation.

SP injection. The SP-induced decrease in BP was significantly inhibited by both FK224 and FK888 (Figure 7). Similarly, $[\text{Sar}]\text{SP}$ significantly ($P < 0.05$) reduced BP by $58.2 \pm 6.9\%$ ($n = 5$). Both FK888 and FK224 significantly ($P < 0.05$) inhibited $[\text{Sar}]\text{SP}$ -induced decreases in BP ($16.6 \pm 13.7\%$ increase with FK888 and $12.2 \pm 4.7\%$ decrease with FK224, $n = 5$ each). In capsaicinized animals, PAF significantly ($P < 0.05$) reduced BP by $48.6 \pm 5.0\%$. Neither FK888 nor FK224 significantly affected this response (reduction of $26.2 \pm 17.0\%$ with FK888 and $39.0 \pm 9.7\%$ with FK224, $n = 5$ each).

During electrical stimulation of the vagus nerves, BP fell continuously (Figure 8). Although FK224 showed a tendency to inhibit the BP-decrease, the effect was not statistically significant ($P = 0.06$ at $1 \mu\text{mol kg}^{-1}$, 3 and 4 min after the start of stimulation). FK888 showed no tendency to any significant inhibitory effect on the BP decrease at either of the doses used or at any of the time points examined.

Figure 7 Effects of FK224 or FK888 on substance P (SP, 1 nmol kg^{-1})-induced decrease in mean carotid artery blood pressure *in vivo* in guinea-pigs. Open column, dimethylsulphoxide (DMSO) + saline; solid column, DMSO + SP (1 nmol kg^{-1}); hatched and cross-hatched columns, effects of FK224 or FK888. All drugs given intravenously. The datum of the control group for FK224 is the average for 2 animals. The other data are expressed as mean for 4–8 animals; s.e.mean shown by vertical bars. * $P < 0.05$; ** $P < 0.01$ vs DMSO + saline.

Figure 8 Effects of FK224 (a) and FK888 (b) on vagus nerve stimulation-induced changes in carotid artery blood pressure *in vivo* in guinea-pigs. Dimethylsulphoxide (DMSO) + sham stimulation (○); DMSO + vagus nerve stimulation (●); effects of FK224 at $0.3 \mu\text{mol kg}^{-1}$ (□) or $1 \mu\text{mol kg}^{-1}$ (▲) in (a), effects of FK888 at $0.05 \mu\text{mol kg}^{-1}$ (■) or $0.2 \mu\text{mol kg}^{-1}$ (Δ) in (b). All drugs given intravenously. Data are expressed as mean for 5 animals; s.e.mean shown by the vertical bars.

Discussion

In the present *in vivo* study, we found that both FK224 (NK_1 and NK_2 dual tachykinin antagonist) (Murai *et al.*, 1992; Morimoto *et al.*, 1992) and FK888 (NK_1 -selective antagonist) (Fujii *et al.*, 1992) inhibited plasma exudation induced in guinea-pig airways by exogenous SP, the selective NK_1 agonist [Sar]SP (Maggi *et al.*, 1990), bilateral vagus nerve stimulation or by inhalation of cigarette smoke. These data extend those of others who found that these antagonists inhibited plasma exudation (as well as bronchoconstriction for FK224) induced by SP, NKA or capsaicin *in vivo* in guinea-pigs (Fujii *et al.*, 1992; Murai *et al.*, 1992). Natural agonist studies (Rogers *et al.*, 1988) indicated that the tachykinin NK_1 receptor was the more important in mediating plasma exudation evoked by tachykinins. Recently, an NK_1 -selective non-peptide antagonist, CP-96,345 (Snider *et al.*, 1991), has been used to demonstrate that neurogenic plasma exudation in rat or guinea-pig airways is mediated predominantly via NK_1 -receptors (Delay-Goyet & Lundberg, 1991; Eglezos *et al.*, 1991; Lei *et al.*, 1992). Our present results provide further evidence for the involvement of NK_1 -receptors in neurogenic plasma exudation, and demonstrate that both FK224 and FK888 are potent tachykinin antagonists *in vivo*. *In vitro* data using pA_2 values indicate that FK888 is approximately 300 times more potent than FK224 as an NK_1 receptor antagonist (Fujii *et al.*, 1992; Morimoto *et al.*, 1992). Our present *in vivo* data for the effect of these antagonists in inhibiting SP-induced plasma exudation demonstrate that FK888 is only 5–10 times more potent than FK224. Similar data have been reported recently (Fujii *et al.*, 1992; Murai *et al.*, 1992). The precise reasons for the discrepancy between

the *in vitro* and *in vivo* data are unclear. One possibility is differences in pharmacokinetics (tissue distribution, bio-availability, metabolism) of the compounds given intravenously, since by intratracheal installation *in vivo* FK888 is 100 times more potent than FK224 (Y. Hirayama, unpublished observations). Alternatively, the discrepancy in potencies may indicate that SP-induced plasma exudation is not mediated exclusively via NK_1 receptors. The involvement of NK_2 receptors in the response is unlikely because the NK_2 -selective agonist, $[\beta\text{-Ala}] \text{MKA}$ (Maggi *et al.*, 1990b), did not induce plasma exudation in the present study. A non-specific action is also unlikely because neither tachykinin antagonist inhibited plasma exudation (or indeed bronchoconstriction or systemic hypotension) induced by PAF. PAF, histamine and sulphidopeptide leukotrienes induce bronchoconstriction and changes in systemic blood pressure (Barnes *et al.*, 1988). Of these, PAF is markedly the most potent in inducing plasma exudation in guinea-pig airways (Evans *et al.*, 1989). However these three mediators have also been found to release SP- and NKA-like immunoreactivity in guinea-pig lungs (Martins *et al.*, 1991), although in the absence of neutral endopeptidase inhibition, PAF releases the least amounts of tachykinin-like immunoreactivity. Thus, PAF was the most appropriate choice of available mediators to determine any non-selectivity of FK888 and FK224 for tachykinin receptor-mediated plasma exudation, bronchoconstriction or hypotension in the present study. In order to exclude the effects of PAF-induced release of tachykinins, we pretreated animals with capsaicin to deplete sensory neuropeptides, including tachykinins, from sensory nerves (Hozler, 1991). The reduced exudative and bronchoconstrictor responses in capsaicized compared with vehicle pretreated animals (albeit not significant) may indicate a small amount of PAF-induced tachykinin release. The complete lack of inhibitory effect of FK888 or FK224 on PAF-induced plasma exudation or bronchoconstriction in capsaicized animals argues against a non-specific action for these antagonists.

The NK_1 -selective antagonist, FK888, showed no significant inhibitory effect on vagus nerve stimulation-induced non-cholinergic bronchoconstriction, although it gave complete inhibition of plasma leakage induced by vagus nerve stimulation in the same animals. In contrast, the dual antagonist, FK224, inhibited the bronchoconstriction. These data indicate that bronchoconstriction *in vivo* induced by endogenously-released tachykinins is mediated predominantly via NK_2 -receptors, which is consistent with *in vitro* data in human bronchus using natural tachykinin potencies (Advenier *et al.*, 1987) and NK_2 antagonist inhibition of contraction of electrically stimulated-guinea-pig bronchi *in vitro* (Maggi *et al.*, 1991b). In the present study the NK_1 -selective agonist, [Sar]SP (Maggi *et al.*, 1990b), also induced significant, although small, bronchoconstriction, a response markedly inhibited by both FK888 and FK224. These data indicate that NK_1 receptors contribute a minor role to bronchoconstriction in the guinea-pig, a suggestion consistent with that of others (Ireland *et al.*, 1991; Maggi *et al.*, 1991b). Our present agonist data indicate the involvement of both NK_1 and NK_2 receptors in tachykinin-induced bronchoconstriction which is in contrast to our observed lack of effect of FK888 on neurogenic non-cholinergic bronchoconstriction. We interpret this latter result as reflecting the relatively minor role for NK_1 receptors in bronchoconstriction and the differences in resistance of SP and [Sar]SP to degradation by endogenous peptidases. [Sar]SP, despite its resistance to peptidase degradation, induced here only a very small increase in airway pressure. Thus, any small intrinsic effect of endogenous SP in inducing bronchoconstriction will be further reduced by enzyme action which makes the involvement of NK_1 receptors in neurogenic bronchoconstriction difficult to demonstrate. The latter suggestion is consistent with the finding *in vitro* that the minor relative contribution of NK_1 receptors to neurogenic bronchoconstriction is increased in the presence of peptidase inhibitors (Maggi *et al.*, 1991b).

Both FK224 and FK888 inhibited hypotension induced by SP, indicating that the vasodilator activity of SP is mediated via NK₁-receptors, a suggestion which is consistent with a recent report using exogenously-administered tachykinins in dogs (Constantine *et al.*, 1991). In contrast, neither compound, at doses that significantly inhibited SP-induced decreases in BP and induced complete inhibition of neurogenic plasma exudation, had any significant inhibitory effect on the decrease in BP induced by vagus nerve stimulation, although FK224 showed a tendency to partial inhibition of the response. This effect of FK224 may be due to an inhibitory effect on bronchoconstriction, which can induce hypoxia with subsequent bradycardia. The contribution of endogenous tachykinins, SP and/or NKA, to neurogenic hypotension would, therefore, appear to be minimal. Other neuropeptides released simultaneously together with SP, including calcitonin gene-related peptide (CGRP) (Saria *et al.*, 1988), may be involved in the hypotensive response. Antidromically released CGRP may affect arterial pressure via a direct action on heart function or via vasodilator activity, which could change blood flow resistance. We also cannot exclude the possibility that adrenergic pathways affected the response. Development of selective and potent CGRP receptor antagonists would serve to elucidate the role of this peptide in neurogenic hypotension.

An axon reflex has been proposed as a mechanism amplifying the chronic inflammation and hyperresponsiveness of asthma (Barnes, 1986), in which neuropeptides released antidromically from sensory C-fibres play a critical role. Increased microvascular permeability with consequent plasma exudation and oedema is a feature of a number of airway diseases including asthma (Rogers & Evans, 1992), although

no evidence is available for induction of plasma exudation by tachykinins in human airways, or about the receptor type(s) involved. Bronchoconstriction may be induced in human airways via NKA-activation of NK₂ receptors (Advenier *et al.*, 1987). Similarly, activation of capsaicin-sensitive sensory nerves in human bronchi induces mucus secretion (Rogers & Barnes, 1989). Natural agonist potencies indicate that NK₁ receptors mediate tachykinin-induced mucus secretion in human airways (Rogers *et al.*, 1989a), although this has not been definitively proven by the use of receptor-selective agonists and antagonists. Nevertheless, the evidence for the involvement of tachykinins in asthma is circumstantial. Tachykinins appear to be important pathophysiological regulators of airway function in guinea-pigs, but their role in human airway disease is substantially unsettled. Similar considerations apply to the receptors involved in the actions of tachykinins in the airways. Data in the species which has been more investigated, the guinea-pig, are not yet confirmed for human airways. Development of selective and potent tachykinin receptor antagonists will be important in understanding the involvement of tachykinins in airway physiology and pathophysiology. If tachykinins are shown to be important in airway pathophysiology, development of potent but non-selective (i.e. dual) tachykinin antagonists may have greater therapeutic potential in asthma and in airway diseases associated with cigarette smoking, including chronic bronchitis.

Note added in proof

Ichinose, M. *et al.* (1992) report that FK224 inhibits bradykinin-induced bronchoconstriction in asthmatics (*Lancet*, **340**, 1248–1251).

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